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Genetic predisposition to lymph node tuberculosis, study of polymorphism of a candidate discomfort: MBL2.

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ABSTRACT

The WHO estimates that between 2000 and 2020, nearly one billion people will be newly infected with TB, 200 million of them develop the disease 35 million will die if no progress is made in the control infection.[1].The identification of genetic factors predisposing to tuberculosis is fundamental to understanding the pathophysiology of this disease and allow the development of new vaccines and therapeutic strategies. Several studies have focused on the gene polymorphism MBL2 [2,3,4] and its association with the pulmonary form of TB. In this work, we will be of interest to the MBL2 gene polymorphism research in a population of 10 children with lymph node tuberculosis.

Keywords : lymph node tuberculosis, MBL2 gene.

INTRODUCTION

Tuberculosis is a communicable infectious disease caused by Mycobacterium tuberculosis, which mainly affects the lungs. The lymph node localization is the most frequent extrapulmonary (up to 40% of extrapulmonary tuberculosis), with predominance in the cervical level (70-90% of cases).Several factors of socio-economic and environmental risks are associated with the development of tuberculosis. [5] However, these factors do not explain several clinical observations such as the development of tuberculosis disease in only 10% of people in contact with TB patients, high concordance among monozygotic twins compared with dizygotic by and ethnic differences in the prevalence and severity of tuberculosis in people living in similar social conditions. The disease expression then results from complex interactions between the bacillus, environmental factors, and factors specific to the host, and it is quite remarkable to note that the vast majority (about 90%) of infected individuals not develop clinical symptoms. Recent publications support the hypothesis of an interaction host / pathogen, these studies provide proof of concept of a continuous spectrum of genetic susceptibility to tuberculosis, from a simple monogenic control complex polygenic inheritance through intermediate effects of major gene. [5]

Several "candidate genes" were studied: NRAMP1 gene, the VDR gene (receiver vitamin D), the IL-12Rb1 gene and the gene MBL2 (Mannose Binding Lectin 2).

MATERIALS AND METHODS

We proceeded to the blood sample, the genomic DNA was extract using phenol-chloroformmethod. Knowledge of the sequence regions that delimit the DNA to be amplified, or target DNA is required. These sequences will be used to synthesize complementary oligonucleotides with a length of 15 to 25 nucleotides in general and which serve to delimit the portion of DNA to be amplified.

Practical realization:

We proceeded to the blood sample, the genomic DNA was extract using phenol-chloroform method.

Knowledge of the sequence regions that delimit the DNA to be amplified, or target DNA is required. These sequences will be used to synthesize complementary oligonucleotides with a length of 15 to 25 nucleotides in general and which serve to delimit the portion of DNA to be amplified.

a).**There. PCR product purification for the reaction sequence:**

Purification of the PCR products is intended to separate the desired amplified sequence of other nucleic acids as well as salts and other components of the reaction medium. It is performed through electrophoresis on agarose gel 2%.

b). **sequencing:**

The sequence reaction is carried out on the thermal cycler and the sequence must be purified on a resin column before being analyzed by Genetic Analyzer.

This step is intended to purify by gel-filtration of the PCR amplification products and the sequence reaction products.

The resin used makes it possible to de-salt the samples and remove unincorporated nucleotides and the PCR primers in excess, which may alter the reading of the sequence. The reading sequence is controlled by a PC platform.

We identified the MBL2 gene sequence which is composed of four exons.

The development of different exons was performed on a gradient thermocycler.

The sequencing results were analyzed and compared with the reference sequences.

RESULTS AND DISCUSSION

1. The reason for consultation: the presence of cervical lymphadenopathy.

2. Age: The average age of patients was 12.5 years (Graph 1).

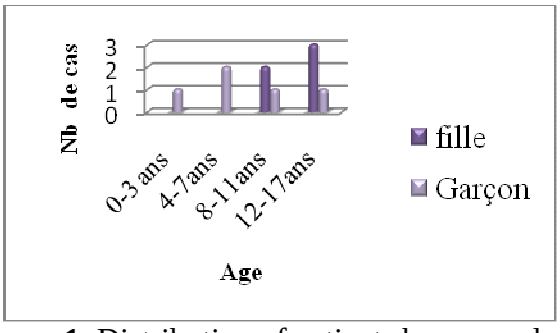

Figure 1: Distribution of patients by age and sex

3. Clinical signs: impairment of the general state with signs of tuberculous impregnation. Weight loss and fever were the most common.

4. TB contagion: shown in one patient at the age of 6 months whose brother had pulmonary tuberculosis.
5. BCG vaccination: all patients received BCG at birth, immunization scar is visible.
6. The ingestion of raw milk was observed in one patient**.**
7. paraclinical explorations included:
a). Laboratory tests that has objectified:
- A VS accelerated in a patient, the NFS showed leukoneutropenia with one child and CRP was normal for all patients.
-A Skin test (TST) tuberculin (Chart 4).

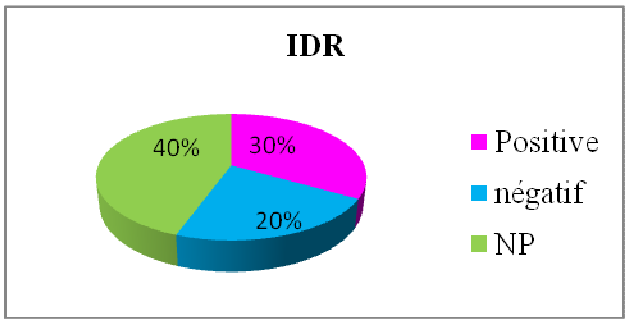

Figure 2 : Résultat de L'IDR de nos patients

b. A radiological assessment includes a chest x-ray was normal in all patients and cervical ultrasonography (Figure 5).

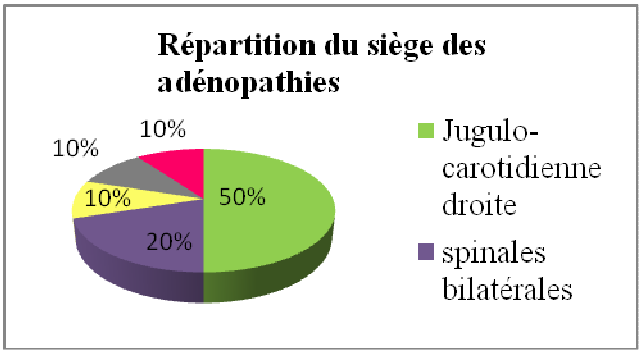

Figure 3: The distribution of lymph nodes according to the seat.

c. Lymph node biopsy with histological examination was performed in all patients revealed the presence of follicular lesions signing caseo-tuberculous lymphadenopathy.

d. Genetic study:

The results of sequencing the gene MBL2 is illustrated in the following tables:

Table 1: genotypic frequency of the gene MBL2
Table 2: Frequency allelic gene MBL2
SNP: Single nucleotide polymorphism.

SNP ou génotype	Patients (n)	Pourcentage(%)
Fréquence génotypique		
Promoteur (rs7096206) (X/Y)		
CC	0	0%
Cg	3	30%
gg	7	70%
5'UTR (rs7095891) (P/Q)		
CC	4	40%
Ct	5	50%
tt	1	10%
Intron1		
CC	3	30%
Ct	6	60%
tt	1	10%
Exon1 rs5030737 (codon 52) (A/D)		
CC	10	10%
Ct	0	0%
tt	0	0%
Exon1 rs1800450 (codon 54) (A/B)		
GG	8	80%
Ga	1	10%
aa	1	10%
Exon1 rs1800451 (codon 57) (A/C)		
GG	10	10%
Ga	0	0%
aa	0	0%
Exon2		
GG	9	90%
Gt	1	10%
tt	0	0%
Exon4 (rs930507)		
CC	0	0%
Cg	3	30%
Gg	7	70%

Table 1: genotypic frequency of the gene MBL2

Allèle	Patients (n)	Pourcentage(%)
Promoteur (rs7096206)		
C	0.15	15%
g	0.85	85%
5'UTR (rs7095891)		
C	0.65	65%
t	0.35	35%
Intron1		
C	0.6	60%
t	0.4	40%
Exon1 rs5030737 (codon 52)		
C	1	100%
t	0	0%
Exon1 rs1800450 (codon 54)		
G	0.85	85%
a	0.15	15%
Exon1 rs1800451 (codon 57)		
G	10	10%
aa	0	0%
Exon2		
G	0.95	95%
t	0.05	5%
Exon4 (rs930507)		
C	0.15	15%
g	0.85	85%

Table 2: Frequency allelic gene MBL2

Discussion:

MBL2 the gene coding for the MBL protein has four exons. The first described mutation G54D, is the most frequently found in the Caucasian population [6]. Two other substitutions are known: G57E and R52C. These three missense mutations type are located in the MBL2 exon1 gene. These amino acid changes are creating structural changes in the collagen domain of the protein and are responsible for a low protein level circulating MBL and a decrease in its biological activity [4,5].

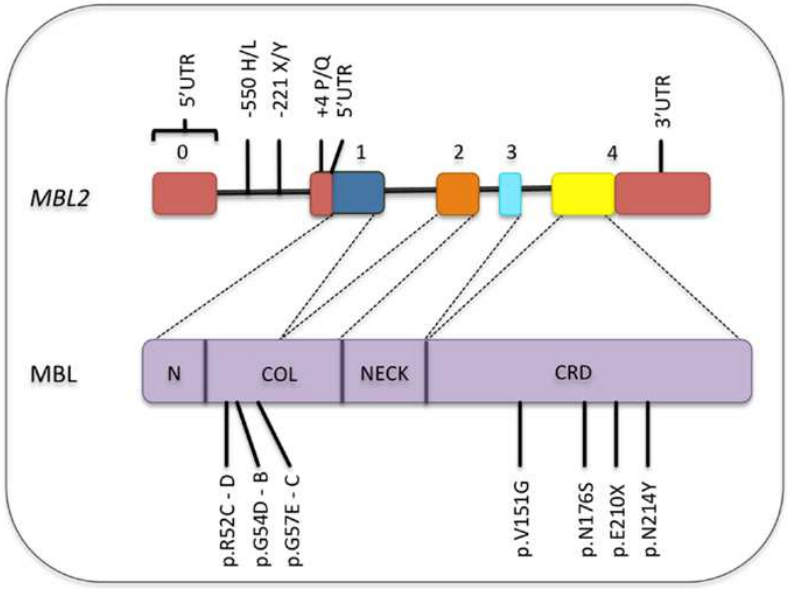

Figure 4: constitution MBL2 MBL gene and protein

Of biallelic polymorphisms or SNP (single nucleotide polymorphism), located in the promoter region of the gene (known as H / L and Y / X) and in the 5 'untranslated region of exon 1 (allele P / Q), are also associated with low MBL synthesis rate. In addition, these SNPs are not randomly distributed in the population, and there is a major imbalance between these liaison alleles [4].

MBL deficiency may result from:
• In the presence of two missense mutations.
• From the combination of two combinations of SNPs associated with a low level of expression of the gene MBL2 (haplotype "low secretory").
• In the presence of a missense mutation and haplotype "low secretor" trans (each carried by a chromosome) [6].
We have undertaken the sequencing of four exons of MBL2 gene and the promoter region and 5 'UTR region and we have highlighted the presence of several variations.

Genotype gg (or YY) of the dual mutated homozygous rs7096206 polymorphism of the promoter region of MBL2 gene was found in 70% of patients, while genotype Cg (or XY) heterozygous was illustrated in 30%. The CC genotype (or XX) wild is absent.

The rare genotype of rs7096206 gg was associated with an increased risk of infections that would be due to a deficiency of MBL [7].

The CC genotype (or PP) of wild rs 7095891 polymorphism 5'UTR of MBL2 gene was found in 40% of patients while the heterozygous genotype Ct (or PQ) was found in 50% of cases. The tt genotype (or QQ) Double homozygous mutant was found in only one patient (10%).

Codon 52 (rs5030737) of the exon 1 of the gene MBL2 showed no variation, the genotype CC (homozygous wild-type) was found in all patients (100%).

Codon 57 (rs1800451) of the exon 1 of the gene MBL2 showed no variation, the GG genotype (homozygous wild-type) was found in all patients, this confirms the perfect linkage disequilibrium between these two codons described in literature [7,8].

The GG genotype (or AA) (wild homozygous) of codon 54 (rs1800450) of exon1 was found in 80% of patients.

While the Ga genotype (or AB) (heterozygous) of codon 54 (rs1800450) of Glycine exon1 resulting change in aspartic acid was found in only one patient (10%).

The genotype AA (or BB) (double mutated homozygous) of codon 54 (rs1800450) of exon1 [GGC→GAC] has been shown in only one patient (10%), this mutation at codon 54 also causes a change the amino acid glycine in the aspartic acid. The consequence of this mutation is to reduce the serum level of MBL protein thus resulting in a non fastening MASP protein (serine protease associated with MBL) essential for the activation of the complement cascade with a default opsonization and infection recurrent bacterial.

Studies have shown reduced levels of MBL protein in subjects with a heterozygous mutation Ga codon 54 of the gene exon1 of MBL2 or undetectable levels in homozygous aa. This can be explained by the increased degradation of the mutated MBL because, as in all field collagen protein, these changes make the protein unable to form a stable secondary structure [9].

Major allelic variants MBL2 gene (codon 54) would be responsible for a decrease in the serum concentration of the protein and might account for a reduction of non-specific immunity in relation to different infectious environments [10].

Exon 2 of MBL2 gene showed a new mutation heterozygous [AGT→ ATT] gene at position [299] in one patient (10%) the resulting amino acid change serine to isoleucine, which might have an impact on the protein structure of MBL2.
Exon 3 of MBL2 gene showed no genotypic variation.
Genotype gg (double mutated homozygous) of the rs930507 polymorphism of the exon 4 of MBL2 gene was found in 70% of the patients while the Cg genotype (heterozygous) was illustrated in 30%. The wild genotype CC is absent.
Given these results, these polymorphisms have a crucial role in the innate immune response leading to susceptibility to glandular tuberculosis.

CONCLUSION

Through this work consisted of ten children with one or more of tuberculous cervical lymphadenopathy, we have highlighted the presence of several polymorphisms in the gene selected MBL2 as a candidate gene or polymorphisms already described in the literature is new variation in the exon2. It would also be interesting to corroborate the probable deficit of MBL protein caused by these polymorphisms and this by serum assay of this protein, it constituerai a vast field of research should be further clinical studies.

Conflict of interest :
No
Author Contributions:
All authors contributed to the realization of the article. All authors have read and approved the final manuscript.

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